Mineralocorticoid Receptor Antagonist Effect on Aldosterone to Renin Ratio in Patients with Primary Aldosteronism

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1   **Mineralocorticoid receptor antagonists effect on Aldosterone to REnin ratio**

2   **for primary Aldosteronism: the MAREA study.**

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Abstract

Purpose: We aimed to evaluate the effect of mineralocorticoid receptor antagonists on aldosterone-to-renin ratio in patients with primary aldosteronism.

Methods: We prospectively enrolled 121 patients with confirmed primary aldosteronism who started a mineralocorticoid receptor antagonist (canrenone) treatment. Eighteen patients (11 with unilateral and 7 with bilateral primary aldosteronism) composed the short-term study cohort and underwent aldosterone, renin and potassium measurement after 2 and 8 weeks of canrenone therapy. The long-term cohort comprised 102 patients (16 with unilateral and 67 with bilateral primary aldosteronism, and 19 with undetermined subtype) who underwent hormonal and biochemical re-assessment after 2 to 12 months of canrenone therapy.

Results: Renin and potassium levels showed a significant increase, and aldosterone-to-renin ratio displayed a significant reduction compared with baseline after both a short and long-term treatment. These effects were progressively more evident with higher doses of canrenone and after longer periods of treatment. We demonstrated that canrenone exerted a deep impact on the diagnostic accuracy of the screening test for primary aldosteronism: the rate of false-negative tests raised to 16.7%, 38.9%, 54.5% and 72.5% after 2 weeks, 8 weeks, 2-6 months and 7-12 months of mineralocorticoid receptor antagonist treatment, respectively.

Conclusions: Mineralocorticoid receptor antagonists should be avoided in patients with hypertension before measurement of renin and aldosterone for screening of primary aldosteronism.

Introduction

Primary aldosteronism (PA) is the most frequent form of endocrine hypertension and it is diagnosed in 5.9% of the general hypertensive population (1) and in 11.2% of patients admitted to hypertension referral centers (2). Patients with PA display an increased risk for cerebrovascular, cardiovascular and renal complications compared with individuals with essential hypertension (3,4).

51 Despite being a highly prevalent and curable form of hypertension, PA remains largely

52 underdiagnosed and undertreated (5,6). According to current guidelines, around 50% of

53 hypertensive individuals display a substantial probability of being affected by PA, and screening

54 should be offered to these patients (7,8). Measurement of plasma renin activity (PRA) and

55 aldosterone concentration (AC), and calculation of the aldosterone-to-renin ratio (ARR) are the

56 mainstay of PA screening work-up. In order to avoid false positive or negative ARR results, the

57 current guidelines recommend to withdraw drugs interfering with renin-angiotensin-aldosterone

58 system (RAAS) before ARR assessment (7,8). In particular, mineralocorticoid receptor antagonists

59 (MRAs), by means of natriuresis and contraction of plasma volume, stimulate RAAS activation,

60 thus renin elevation and ARR reduction (9). Because of the potential of MRA-related false negative

61 results, present recommendations are to stop MRAs for up to 4 to 6 weeks before screening test for

62 PA is performed (7,8). Nevertheless, recent reports suggested that diagnosis and subtyping of PA

63 might be achieved in selected patients with florid PA in whom MRA withdrawal is not feasible

64 because of uncontrolled hypertension and/or severe hypokalemia, and in whom renin levels remain

65 suppressed despite ongoing MRA treatment (10-12).

66 From another standpoint, Hundemer et al recently reported that patients with PA treated with an

67 MRA and displaying increase of renin activity showed a reduced risk of mortality, cardiovascular

68 events and incident atrial fibrillation compared with PA patients whose PRA remained low despite

69 MRA therapy (13,14). Therefore, elevation of renin levels might represent a biochemical marker of

70 adequate mineralocorticoid receptor (MR) blockade (15).

71 The aim of our study was to investigate the effects of MRA treatment on the accuracy of PA

72 screening test in a cohort of patients with confirmed PA and the time and dose-dependent effects of

73 MRA on ARR.

74

75 **Materials and Methods**

76 **1. Study Design and Patients**

3

The research protocol was approved by our local Ethics Committee. All recruited patients gave written informed consent.

Patients referred to the University of Torino Hypertension Center were prospectively enrolled. Inclusion criteria were: (1) age between 18 and 75 years; (2) confirmed diagnosis of PA; (3) medical treatment with an MRA (canrenone). Exclusion criteria were: (1) concomitant intake of RAAS-interfering drugs, such as beta-blockers, diuretics, angiotensin-converting enzyme inhibitors (ACE-Is) and angiotensin II-receptor blockers (ARBs); (2) patient refusal.

The study cohort was composed of two subgroups of patients. The first one (long-term MRA treatment group) comprised patients with PA not eligible for adrenalectomy (ADX) because of (1) bilateral primary aldosteronism (BiPA), (2) delayed or refused surgery in those affected by unilateral primary aldosteronism (UPA), or (3) patients with undetermined subtype diagnosis. PRA, AC and ARR were assessed at baseline and after 2 to 12 months of MRA treatment at follow-up visit to evaluate the treatment efficacy. The second group (short-term MRA treatment group) was composed of (1) patients with both PA subtypes who initiated MRA treatment because of surgery ineligibility, and (2) patients affected by UPA that started MRA awaiting ADX. Plasma laboratory assessments were performed after 2 and 8 weeks of MRA therapy.

We prospectively recruited 121 patients with confirmed PA who started MRA treatment (Figure 1). One patient of the short-term cohort was lost to follow-up and excluded from the analysis. The long-term MRA treatment subgroup was composed of 102 patients (67 affected by BiPA, 16 affected by UPA and 19 PA patients with an undetermined subtype diagnosis), while the short-term MRA treatment subgroup comprised 18 patients (7 with BiPA and 11 with UPA awaiting surgery). Baseline clinical and biochemical data of the included patients are reported in Table 1. Blood pressure measurements according to European Society of Hypertension guidelines (16), clinical evaluation, and biochemical assessment of PRA, AC and potassium levels were obtained for all patients at follow-up (after 2 and 8 weeks of MRA therapy in the short-term treatment group, and after 2 to 12 months for the long-term group) (Supplementary Tables 1-2) (17).

4

103   To estimate medication adherence and its potential interference with ARR results, therapeutic drug

104   monitoring (TDM) of canrenone was performed in a proportion of patients (37.3% of the long-term

105   cohort and 100% of the short-term cohort, both after 2 and 8 weeks of therapy).

106

107   **2. Diagnosis of Primary Aldosteronism**

108   PA was diagnosed according to current Endocrine Society and European Society of Hypertension

109   recommendations (7,8). Briefly, screening testing was performed after withdrawal of all RAAS-

110   interfering antihypertensive drugs (at least for 2 weeks for beta-blockers, ACE-Is and ARBs, and 4

111   weeks for diuretics; no patients were under MRA) and non-interfering drugs (such as calcium-

112   channel blockers or alpha-1 blockers) were used to control blood pressure, if necessary. The

113   screening test was considered positive if AC $\geq$ 10 ng/dL and ARR $\geq$ 30 ng/dL/ng/mL/h. PA

114   diagnosis confirmation was obtained by seated saline infusion test (SSIT) or captopril challenge test

115   (CCT), whenever the former was contraindicated. Subtype diagnosis was performed by contrast-

116   enhanced computed tomography of the adrenal glands and unstimulated and/or ACTH-stimulated

117   adrenal vein sampling (18).

118

119   **3. Biochemical measurements**

120   Plasma renin activity and aldosterone concentration were measured as previously described (1).

121   TDM of canrenone was performed on urine samples obtained at the same time of blood collection

122   for follow-up assessments, and biochemical analyses were run as previously described (19).

123

124   **4. Statistical Analysis**

125   IBM SPSS Statistics version 26.0 (IBM Corp, Armonk, New York) and GraphPad Prism version

126   8.0.0 (GraphPad Software, San Diego, California) were used for statistical analyses. Data were

127   expressed as mean $\pm$ standard deviation for continuous variables with a normal distribution, or

128   median [interquartile range] for variables with a non-normal distribution. Normally distributed data

129   were compared with Student *t* test for independent variables or with one-way ANOVA with

130   Bonferroni post hoc test in case of unpaired samples, and with Student *t* test for dependent variables

131   or repeated measures one-way ANOVA with Holm-Sidak post hoc test in case of matched samples.

132   Non-normally distributed data were compared with Mann–Whitney U test or with Kruskal-Wallis

133   test and Dunn post hoc test in case of independent samples, and with Friedman test with Dunn post

134   hoc test in case of matched samples. Categorical variables were expressed as absolute number and

135   percentage, and compared with χ^2 test or Fisher exact test, as appropriate. P values < 0.05 were

136   considered statistically significant.

137

138   **Results**

139   **1. Short-term effects of MRA on ARR in patients with PA**

140   Nineteen patients with PA were recruited in the short-term study cohort. One patient was lost to

141   follow-up after the 2-week assessment and excluded from the analysis (this patient also resulted not

142   compliant to canrenone therapy when evaluated with TDM). Of the 18 patients who underwent the

143   short-term follow-up, 11 (61.1%) were affected by UPA and 7 by BiPA (38.9%) (Table 1). As

144   expected, systolic and diastolic blood pressure values were significantly reduced by MRA treatment

145   (Supplementary Table 1) (17). Potassium levels were significantly higher after both 2 and 8 weeks

146   (+8.6% and +14.3%, respectively) of canrenone therapy compared with baseline values (p=0.011)

147   (Figure 2A). Similarly, PRA levels showed a significant 56.7% and 140% increase after 2 and 8

148   weeks of MRA treatment, respectively (p<0.001) (Figure 2B). Follow-up AC was not significantly

149   different compared with baseline (Figure 2C). As a result, ARR values were reduced by MRA

150   treatment (Figure 2D), thus leading to an increase in the rate of false-negative screening tests

151   (p=0.010) (Figure 3A). The rate of false negative tests was 16.7% (3/18) after 2 weeks and 38.9%

152   (7/18; p=0.008) after 8 weeks of canrenone therapy (Figure 3A, Supplementary Table 1) (17).

153

154   **2. Long-term effects of MRA on ARR in patients with PA**

The long-term MRA treatment cohort comprised 102 patients: 16 affected by UPA (15.7%), 67 affected by BiPA (65.7%) and 19 (18.6%) patients with undetermined subtype (Table 1). Clinical and biochemical follow-up was performed after 2 to 12 months of canrenone therapy. To stratify the long-term effects of MRA on RAAS activity, this subgroup of patients was further divided according to the length of follow-up (2 to 6 months and 7 to 12 months). Blood pressure was reduced (p<0.001) (Supplementary Table 2) (17) and potassium levels significantly increased by MRA treatment (p<0.001) (Figure 4A). Of note, the majority of potassium values at follow-up (95%) fell within the normal range. MRA treatment led to a significant increase of PRA values both at 2-6 months (+315%) and 7-12 months (+1050%) compared with baseline (p<0.001) (Figure 4B). Canrenone effect on PRA levels was progressively more evident over time, with PRA values after 7-12 months significantly higher than values after 2-6 months (p=0.015). (Figure 4, Supplementary Table 2) (17). AC remained elevated and was not significantly affected by long-term therapy with canrenone (Figure 4C). ARR was significantly reduced by MRA long-term treatment (-76% and -90.1% after 2-6 and 7-12 months, respectively; p<0.001) (Figure 4D). The rate of false-negative tests considerably increased to 54.5% (18/33) and 72.5% (50/69) after 2-6 and 7-2 months of MRA treatment, respectively (p<0.001) (Figure 3B; Supplementary Table 2) (17).

3. Effects of different MRA doses on ARR in patients with PA

We also evaluated the effect of different doses of canrenone on the ARR. Potassium levels and PRA showed a significant progressive increase compared with baseline in patients treated with increasing MRA doses (p< 0.001) (Figure 5A-B) (Supplementary Table 3) (17). In particular, PRA levels showed an increase of 75% to 1650% compared with baseline in patients treated with 12.5 mg to 100 mg/day of canrenone, respectively. Despite a moderate increase in AC levels (Figure 5C), ARR values progressively decreased with increasing MRA doses (-52.9% to -92.4%) (Figure 5D).

Accordingly, we observed a progressive increase in the rate of false-negative screening tests with

180 higher doses of canrenone, up to 86.7% in patients with PA treated with 100 mg/day of canrenone

181 (Figure 3C; Supplementary Table 3) (17).

182

183 **4. Effects of drug adherence on ARR**

184 Canrenone TDM was performed on a proportion of patients to evaluate treatment compliance and

185 effects of non-adherence on biochemical parameters. Seventy-five TDM tests were performed: 1

186 patient from the short-term cohort was lost to follow-up and excluded from the analysis (urinary

187 canrenone was absent in this patient). Of the remaining 74 TDM tests, 38 were performed on

188 patients of the long-term cohort and 36 in patients of the short-term cohort (all patients were

189 evaluated after 2 and 8 weeks of MRA therapy). TDM showed non-adherence to canrenone in

190 12.2% of cases (9/74). We observed a significantly lower PRA (p=0.010) and markedly higher

191 ARR values (p=0.003) in patients with a negative TDM compared with fully compliant patients

192 (Supplementary Table 4) (17).

193

194 **Discussion**

195 The present study demonstrated a time and dose effect of MRA therapy on the ARR and a deep

196 impact on the diagnostic accuracy as a screening test for PA.

197 Current guidelines recommend to withdraw antihypertensive medications potentially affecting renin

198 and/or aldosterone levels before the performance of screening test for PA, to prevent false-negative

199 or -positive results (7,8). MRAs induce volume contraction and increase of potassium levels,

200 leading to RAAS activation with renin elevation and ARR reduction (9). Because of the potential of

201 false negative PA screening tests, present recommendations are to stop MRAs for up to 4 to 6

202 weeks beforehand (7,8).

203 Some studies displayed the possibility of subtype diagnosis determination by adrenal vein sampling

204 in patients with florid phenotypes, even without MRA withdrawal (10,11), as long as renin levels

205 are still suppressed before the procedure (20). A recent study performed in 42 patients (32 with

206 UPA and 10 with BiPA) reported that 1-month therapy with the MRA canrenone did not interfere

207 significantly with ARR values and with interpretation of the screening (2/42 false negative results,

208 5%); after addition of olmesartan to canrenone for further 4 weeks the rate of false negative

209 diagnoses increased to 8/34 (23.5%) (12). From these data it was not possible to determine if the

210 effect on the ARR after two months was due to the addition of olmesartan or the longer duration of

211 canrenone therapy or both.

212 The aim of the present study was to investigate the effects of MRA treatment on ARR in patients

213 with confirmed PA, and the impact on the accuracy of the screening test.

214 We observed a significant increase of PRA levels already after 2 weeks of MRA therapy and this

215 effect was further magnified by the duration of the treatment up to 12 months. Since aldosterone

216 levels were only marginally affected, ARR displayed a progressive decrease depending on the

217 duration of the MRA therapy. As a result, the rate of false-negative screening tests increased from

218 16.7 to 72.5% after 2 weeks to 12 months of therapy. As expected, the ARR also decreased

219 progressively with the increase of MRA dosage from 30.8% with 12.5 mg of canrenone to 86.7%

220 with 100 mg/day.

221 Our results are in agreement with previous studies (21-25), showing a significant and early increase

222 in PRA levels in patients with PA treated with MRA, resulting in a significant increase in the rate of

223 false-negative screening tests.

224 Even though these effects were evident after just two weeks of MRA therapy, the reduction of ARR

225 appeared to be progressive and incremental over time (21,25). Several explanations were proposed

226 for the gradual increase of renin levels during MRA treatment. First, complete abrogation of the

227 negative feed-back of chronic hyperaldosteronism on RAAS activity might take some time to be

228 observed: the increase of renin release caused by tubuloglomerular feedback activation, in turn

229 driven by MRA-related volume depletion, might be apparent over time (26). Second, canrenone

230 might require a discrete period of time to fully exert its MR blockade effects for the relatively lower

231 affinity for the mineralocorticoid receptor compared with spironolactone (27).

9

232 Discrepancies between our findings and results from the EMIRA study (12) might be potentially

233 related to its design: persistent suppression of renin might result from the limited time interval (1

234 month) between MRA initiation and hormonal follow-up, and the recruitment of patients affected

235 by florid PA phenotype requiring high doses of MRA and prolonged treatment to achieve complete

236 MR blockade.

237 We also investigated MRA drug adherence in a proportion of recruited patients, demonstrating

238 12.2% of absent compliance to the treatment. This is not surprising since partial/total non-adherence

239 was observed in 20-60% of patients with hypertension (28). As expected, PRA levels were

240 significantly lower and ARR was significantly higher in non-adherent patients compared with fully

241 compliant. We may speculate that part of the lack of the effect of MRA therapy on renin levels in

242 some patients with PA might be due variable degrees of reduced treatment compliance.

243 Hundemer et al recently demonstrated that increase of renin levels was related to significant

244 reduction in mortality risk, cardiovascular events rate and atrial fibrillation incidence in patients

245 with PA on MRA treatment (13,14). Renin elevation and ARR reduction might then serve as

246 biochemical markers of adequate MR blockade and potential predictors of favorable clinical

247 outcome (15).

248 Even though our study design and the limited duration of our follow-up were not appropriate to

249 investigate clinical outcomes of PA patients treated with MRAs, we believe our results are

250 supportive of some important insights. First, renin levels should be monitored and MRA treatment

251 up-titrated by the physician to reach the required dose not only to control hypertension and

252 hypokalemia but also to obtain increase of renin levels. Second, TDM may be considered to assess

253 patient compliance to MRA treatment and to guide therapy optimization when renin levels remain

254 suppressed. Lastly, PRA and ARR re-assessment should be performed after an appropriate time

255 span from MRA initiation in order to allow the completion of MR blockade effects and

256 subsequently adjust antihypertensive therapy whenever necessary.

257

258 The current study has some limitations. We did not recruit a control group of patients with PA on

259 antihypertensive treatment without MR blockade to evaluate potential MRA-independent ARR

260 modifications over time. We also did not assess MRA compliance in the whole study cohort,

261 although our TDM estimation might suggest that drug adherence was around 88%, thus likely not

262 interfering our conclusions on ARR assessments. Finally, in the long-term cohort the time of the

263 follow-up and the dose of MRA were non standardized and left to the clinicians' judgement:

264 however, this represents a "real life" situation of what happens during the long-term medical

265 treatment of patients with PA.

266

267 In conclusion, the results of the present study indicate that MRAs display a marked and rapid effect

268 on ARR and therefore, the screening test should be performed after appropriate withdrawal of

269 MRAs. Moreover, our findings support that renin elevation should be used as a biochemical marker

270 of efficient MR blockade.

271

272 **Data Availability**

273 Some or all datasets generated during and/or analyzed during the current study are not publicly

274 available but are available from the corresponding author on reasonable request. Supplemental

275 materials (17) are available at the following link:

276 https://github.com/CentroIpertenUnito/MAREA/raw/main/Supplemental%20materials.pdf.

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Legends for Figures and Tables

Figure 1 – Study design and flowchart of study cohort. Abbreviations: MRA, mineralocorticoid receptor antagonist; PA, primary aldosteronism; RAAS, renin-angiotensin-aldosterone system.

Figure 2 – Short-term effects of MRA on potassium and hormonal parameters in patients with PA. Potassium levels (K+) (**Panel A**), plasma renin activity (PRA) (**Panel B**), aldosterone concentration (AC) (**Panel C**) and aldosterone-to-renin ratio (ARR) (**Panel D**) after 2 and 8 weeks (W) of mineralocorticoid receptor antagonist (MRA) therapy compared with baseline (B). Shadowed area indicates normal values for potassium, unsuppressed renin for PRA (> 1 ng/mL/h) and negative screening test for ARR (< 30 ng/dL/ng/mL/h). Solid horizontal lines represent median [IQR] for PRA, AC and ARR, and mean $\pm$ SD for potassium levels. Levels of significance are reported as follows: $0.01 < p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

Figure 3 – Screening test results in patients with PA under MRA treatment. Rate of false negative screening tests for primary aldosteronism (PA) (in grey) after 2 and 8 weeks (W) (**Panel A**) and after 2-6 and 7-12 months (M) of canrenone therapy (**Panel B**). Rate of false negative screening tests for primary aldosteronism (PA) (in grey) with different doses of canrenone (12.5 to 100 mg/day) (**Panel C**).

Figure 4 – Long-term effects of MRA on potassium and hormonal parameters in patients with PA. Potassium levels (K+) (**Panel A**), plasma renin activity (PRA) (**Panel B**), aldosterone concentration (AC) (**Panel C**) and aldosterone-to-renin ratio (ARR) (**Panel D**) after 2-to-6 months (2-6 M) and 7-

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383 to-12 months (7-12 M) of mineralocorticoid receptor antagonist (MRA) therapy compared with

384 baseline (B). Shadowed area indicates normal values for potassium, unsuppressed renin for PRA (>

385 1 ng/mL/h) and negative screening test for ARR (<30 ng/dL/ng/mL/h). Solid horizontal lines

386 represent median [IQR] for PRA, AC and ARR, and mean ± SD for potassium levels. Levels of

387 significance are reported as follows: $0.01 < p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

388

389 **Figure 5** – Effects of different MRA doses on ARR in patients with PA. Potassium levels (K+)

390 (**Panel A**), plasma renin activity (PRA) (**Panel B**), aldosterone concentration (AC) (**Panel C**) and

391 aldosterone-to-renin ratio (ARR) (**Panel D**) after different doses (12.5 to 100 mg/day) of

392 mineralocorticoid receptor antagonist (MRA) therapy compared with baseline (B). Shadowed area

393 indicates normal values for potassium, unsuppressed renin for PRA (> 1 ng/mL/h) and negative

394 screening test for ARR (<30 ng/dL/ng/mL/h). Solid horizontal lines represent median [IQR] for

395 PRA, AC and ARR, and mean ± SD for potassium levels. Levels of significance are reported as

396 follows: $0.01 < p < 0.05$ (*); $p < 0.01$ (**); $p < 0.001$ (***).

397

398 **Table 1** - Values are mean ± SD, median [IQR], or absolute number (%). Abbreviations: AC,

399 aldosterone concentration; ARR, aldosterone-to-renin ratio; BiPA, bilateral primary aldosteronism;

400 DBP, diastolic blood pressure; DDD, daily defined dose; MRA, mineralocorticoid receptor

401 antagonist; PA, primary aldosteronism; PRA, plasma renin activity; SBP, systolic blood pressure;

402 UPA, unilateral primary aldosteronism.

Table 1 – Clinical and biochemical parameters of patients.

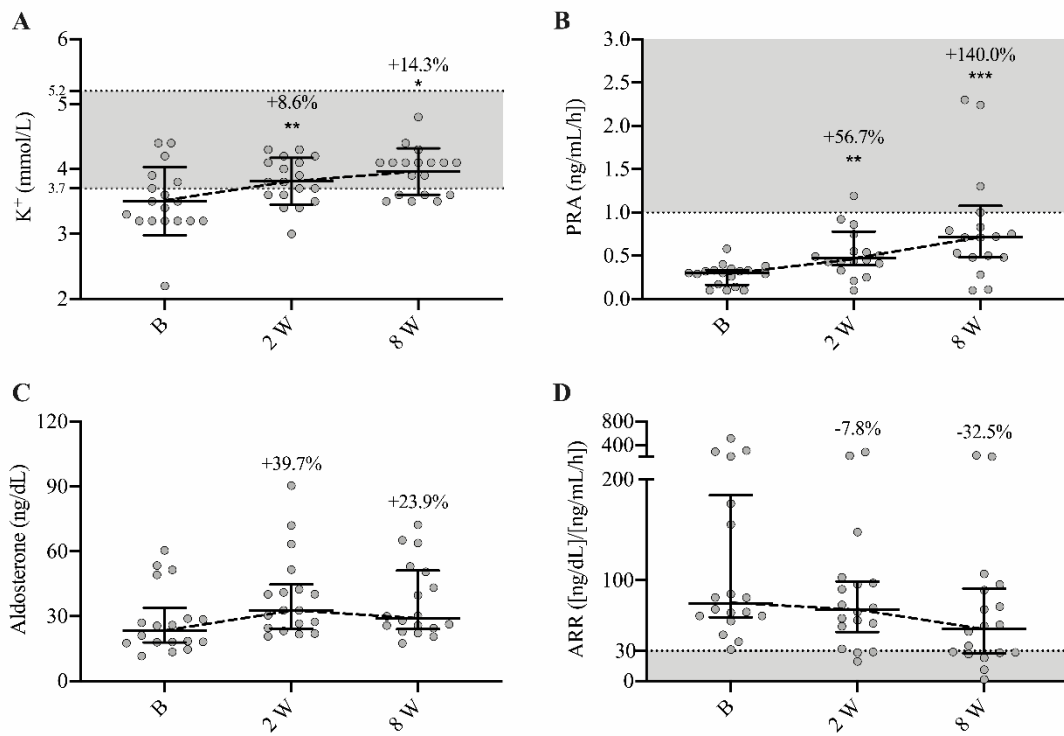| Entire Cohort	n=120	Short-term Cohort	n=18	Long-term Cohort	n=102
Age (years)	50 ± 9.3	Age (years)	54 ± 10.2	Age (years)	50 ± 9.1
Sex (male)	75 (62.5)	Sex (male)	16 (88.9)	Sex (male)	59 (57.8)
Subtype Diagnosis		Subtype Diagnosis		Subtype Diagnosis	
UPA	27 (22.5)	UPA	11 (61.1)	UPA	16 (15.7)
BiPA	74 (61.7)	BiPA	7 (38.9)	BiPA	67 (65.7)
SBP (mmHg)	156 ± 20.6	SBP (mmHg)	150 ± 15.7	SBP (mmHg)	157 ± 21.2
DBP (mmHg)	97 ± 12.3	DBP (mmHg)	88 ± 9.3	DBP (mmHg)	98 ± 12.1
DDD	2.0 [1.0; 3.0]	DDD	3.0 [2.5; 3.3]	DDD	2.0 [1.0; 3.0]
Potassium (mmol/L)	3.7 ± 0.55	Potassium (mmol/L)	3.5 ± 0.53	Potassium (mmol/L)	3.7 ± 0.55
PRA (ng/mL/h)	0.28 [0.10; 0.40]	PRA (ng/mL/h)	0.30 [0.16; 0.34]	PRA (ng/mL/h)	0.20 [0.10; 0.40]
AC (ng/dL)	28.1 [18.3; 37.0]	AC (ng/dL)	23.4 [17.9; 34.0]	AC (ng/dL)	29.0 [19.9; 37.1]
ARR (ng/dL/ng/mL/h)	119 [66; 178]	ARR (ng/dL/ng/mL/h)	77 [63; 184]	ARR (ng/dL/ng/mL/h)	121 [67; 179]
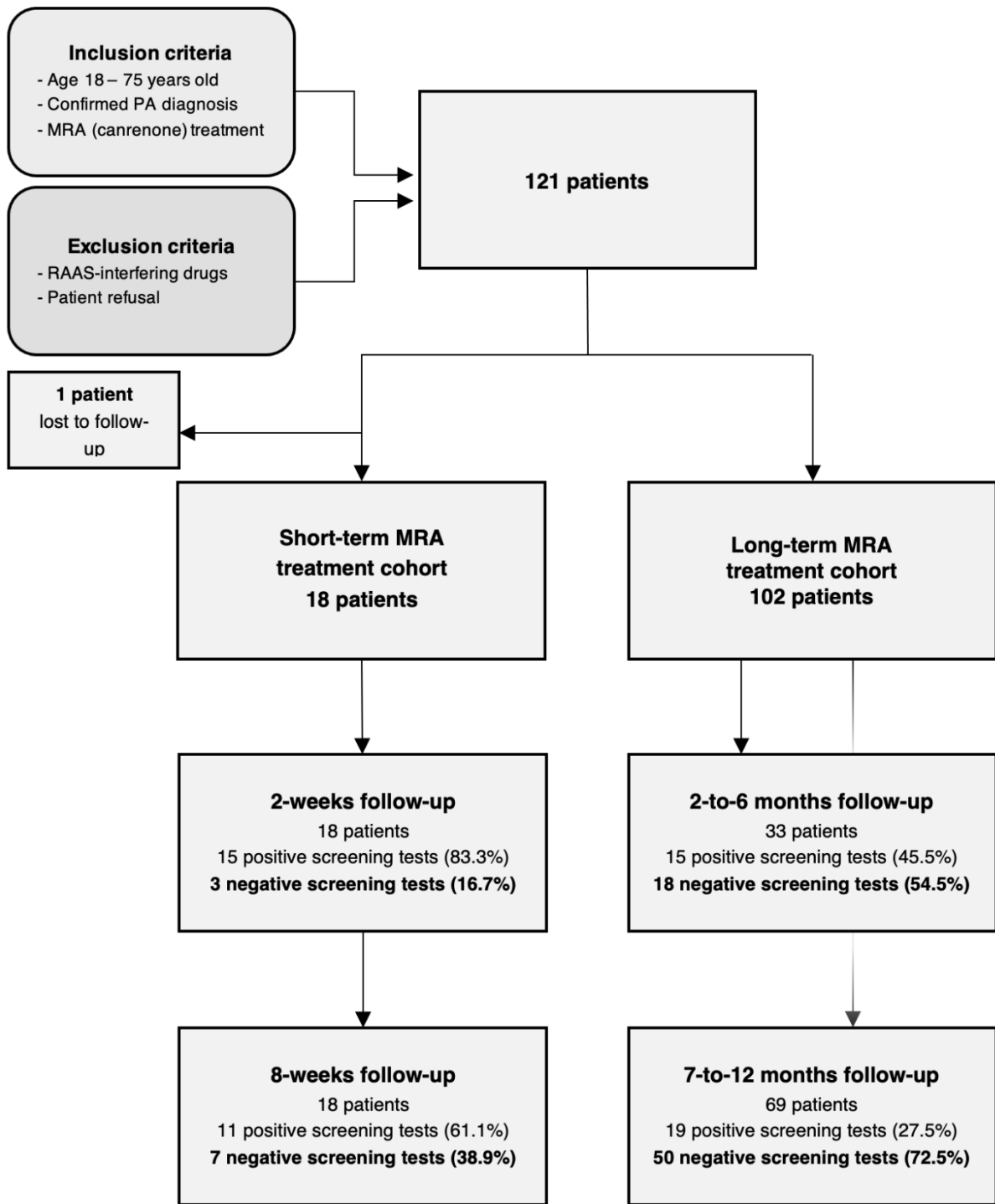
Figure 1
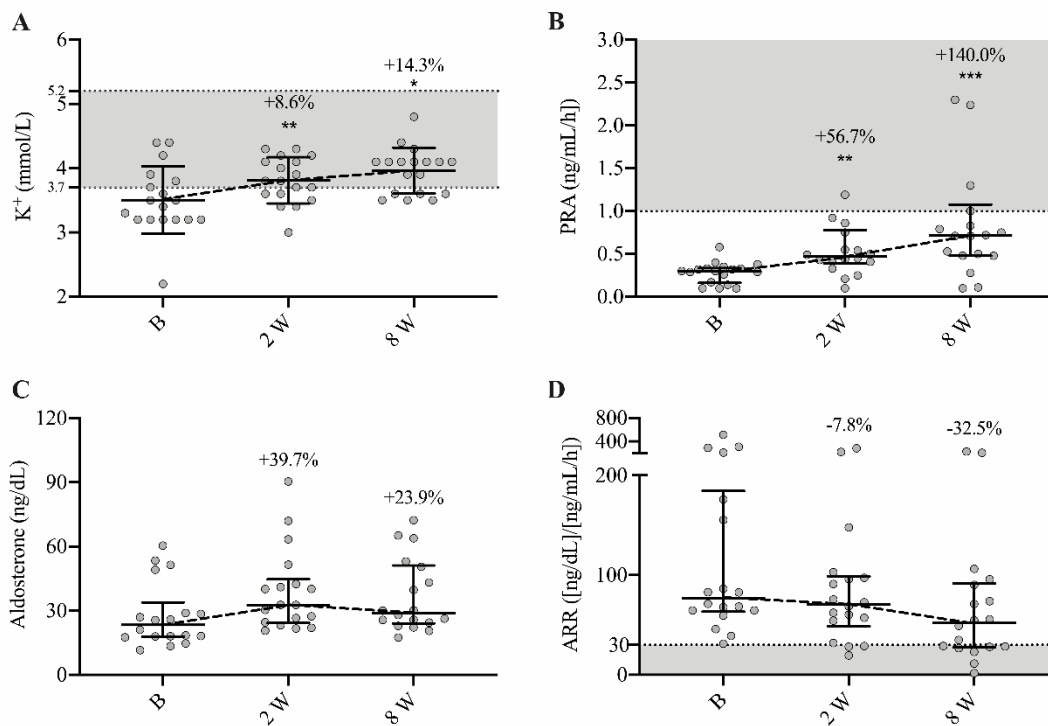
Figure 2

Figure 3

Figure 4

Figure 5